Available online at www.sciencedirect.com

SCIENCE DIRECT®

Virology 327 (2004) 242–248

VIROLOGY

www.elsevier.com/locate/yviro

Cross-reactive pseudovirus-neutralizing anti-envelope antibodies coexist with antibodies devoid of such activity in persistent hepatitis C virus infection

Roberto Burioni[a,*], Nicasio Mancini[a,b], Silvia Carletti[b], Mario Perotti[b], Antonella Grieco[a], Filippo Canducci[b], Pietro E. Varaldo[a], Massimo Clementi[b]

[a]*Istituto di Microbiologia, Facoltà di Medicina e Chirurgia, Università di Ancona, 60020 Ancona, Italy*
[b]*Laboratorio di Microbiologia, Diagnostica e Ricerca San Raffaele, IRCCS Istituto Scientifico San Raffaele, Università "Vita-Salute San Raffaele", 20132 Milan, Italy*

Received 1 March 2004; accepted 18 June 2004
Available online 7 August 2004

Abstract

Most RNA viruses have evolved mechanisms to avoid neutralizing antibody responses, and it is generally believed that variability of envelope-encoding regions is the major molecular basis of this phenomenon. However, it has been hypothesized that other mechanisms can be involved. Recent experimental data indicate that in hepatitis C virus (HCV) infection, the anti-envelope humoral response includes cross-reactive antibody clones able to neutralize vesicular stomatitis virus (VSV) pseudotypes containing HCV E1 and E2 glycoproteins (HCV/VSV pseudotype) as well as other clones devoid of such activity. In this work, we demonstrate that natural infection with a large variety of HCV isolates belonging to different genotypes elicits HCV/VSV pseudotype-neutralizing cross-reactive anti-envelope antibodies together with clones unable to neutralize this pseudovirus. This was shown by designing a novel strategy for quantitation of serum antibodies binding selectively to single viral cross-reactive conformational epitopes. These data can be useful not only for a better understanding of the virus–host interplay in important viral diseases, but also for the development of an effective anti-HCV vaccine.

Keywords: Hepatitis C virus; Persistent infection; Anti-HCV humoral response; Quantitation of antibody subpopulations

Introduction

Hepatitis C virus (HCV) infection is a major health problem affecting 170 million people, or 3% of the world population (Lauer and Walker, 2001). Unfortunately, its most striking feature is the tendency toward chronicity (Cerny and Chisari, 1999) associated with continuous viral replication (Alter et al., 1992), reflecting the inability of the immune response to clear the infection completely in the majority of patients.

Humoral immunity directed against HCV antigens is readily detected, and the demonstration of specific anti-HCV antibodies is the basis of serologic diagnosis of infection (Alter et al., 1989); however, the role of antibodies in protecting from viral infection or in determining disease progression is still unclear. Studies in chimpanzee (Farci et al., 1992), the only available HCV model, and in poly-transfused thalassemic children (Lai et al., 1994) have demonstrated that even in the presence of a high titer of anti-HCV antibodies, a new challenge of convalescent hosts with HCV results in reinfection. In contrast to these findings, there is in vivo and in vitro evidence that HCV infection gives rise to a neutralizing response (Bjoro et al., 1994; Farci et al., 1994; Zibert et al., 1995) and that an important viral target of these antibodies is the E2 envelope glycoprotein. Finally, prophylactic administration of anti-

* Corresponding author. Istituto di Microbiologia, Facoltà di Medicina e Chirurgia, Università di Ancona, Via Conca, 60020 Ancona, Italy. Fax: +39 071 5964850.
E-mail address: robertoburioni@yahoo.com (R. Burioni).

0042-6822/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2004.06.042

HCV antibodies-containing immunoglobulin preparations to individuals at risk has resulted in protection from sexually transmitted infection (Piazza et al., 1997).

A contribution to the knowledge of the anti-HCV/E2 immunoresponse has come from the dissection of the humoral response against this antigen. Cloning of the IgG1/k immune repertoire of an HCV-infected patient in phage display combinatorial vectors and the subsequent generation of recombinant monoclonal Fab fragments representing discrete parts of the human response to a real in vivo infection (Burioni et al., 1998c, 2001b; Plaisant et al., 1997) demonstrated that, at least when analyzed in terms of its ability to neutralize viral pseudotypes bearing HCV envelope glycoproteins on their surface, the immunoresponse is highly heterogeneous, as clones endowed with very strong activity coexist with clones that are totally devoid of it (Burioni et al., 2002). The utilization of these Fabs in defining human B cell epitopes on HCV/E2 allowed to demonstrate the presence of at least four major epitopes, only two of which are recognized by pseudovirus-neutralizing Fabs, suggesting that these antibody fragments interact with a region of E2 indispensable for viral functions. A crucial issue is to understand whether these antibodies are reacting against conserved regions of the viral envelope. In fact, the Fabs were selected in a way that should have privileged cross-reactive epitopes; more in detail, they were obtained by cloning the Fab-coding genes from a patient infected with an HCV of a genotype (1b) different from the one of the protein used for selection (1a) (Lesniewski et al., 1995; Ogata et al., 1991). Given the huge E2 heterogeneity among HCV isolates of different genotypes, it is very likely that these Fabs are directed against conserved regions of envelope glycoproteins. Furthermore, our experiments clearly demonstrated that all these Fabs react with E2 of the 1a genotype and at the same time immunoprecipitate pseudoviruses constructed with E2 of the 1b genotype (Bugli et al., 2001; Burioni et al., 2002). A very important issue for a proper comprehension of the virus–host interplay at this stage is to determine whether similar antibodies are elicited by infections caused by HCV strains of different genotypes.

To study this aspect, using the epitope map generated in previous work (Bugli et al., 2001), we devised a novel method for the indirect serum measurement of antibodies directed against the different epitopes of E2 recognized by our anti-HCV/E2 human monoclonal recombinant Fabs: the determination of the Fab Inhibition Titer (FIT). The quantitation strategy relies on the demonstration of the inhibition of binding of FLAG-labeled human recombinant Fabs (FLAG-Fabs) to bound HCV/E2 antigen using an enzyme-linked immunoassay (Fig. 1). The system was evaluated using mock sera containing known amount of epitope-specific IgG1 human monoclonal antibodies. The method is shown to be simple, reproducible, easy to perform in an ELISA format, and can help elucidate the role of the antibodies directed against the different parts of the E2 molecule.

Results and discussion

The appropriate FLAG-Fab concentration employed in the assay was determined for each FLAG-Fab and was the one giving approximately 50% of maximum reading,

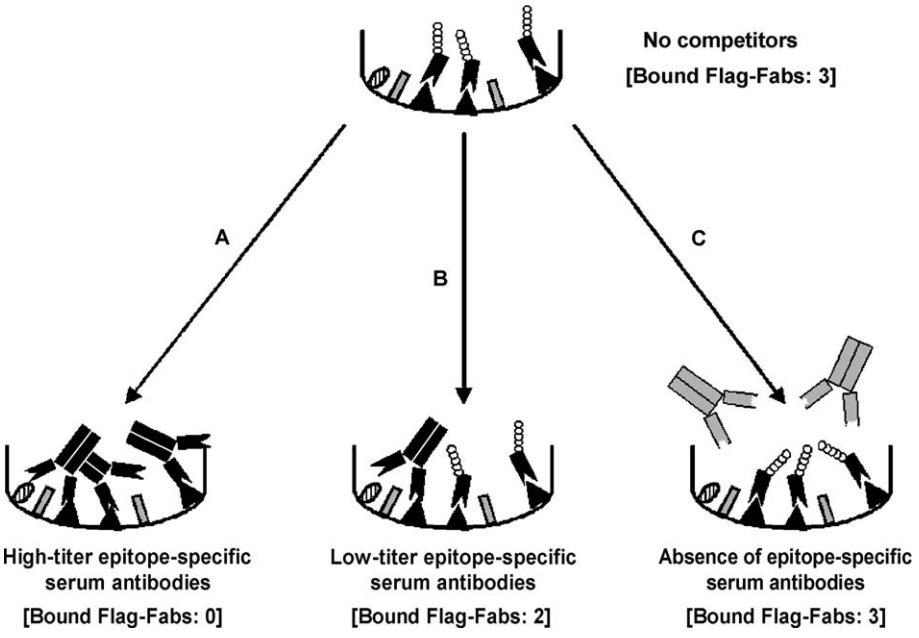

Fig. 1. Schematic representation of FIT. The upper portion of the scheme portrays the specific binding of three probes (FLAG-Fabs) to a single epitope (black triangle) of an antigen in the absence of any competing agent. In the lower section, the binding of the same FLAG-Fabs in three different conditions is depicted. In A and B, probe binding is inversely affected by the titer of epitope-specific serum antibodies (black Igs); in C antibodies from an HCV-negative serum do not exert any influence on the amount of bound probes.

corresponding with 5 μg/ml (e8-FLAG), 2 μg/ml (e20-FLAG), and 10 μg/ml (e137-FLAG). Mean optical densities (ODs) taken as reference values (and relevant standard deviations) were 1.1 (±0.15) for e8-FLAG, 1.2 (±0.10) for e20-FLAG, and 1.0 (±0.10) for e137-FLAG. Determination of FIT was performed on 10 HCV-negative sera; the value obtained was consistently <1:10, the upper detection limit of our test, indicating that no inhibition occurred in the absence of specific anti-HCV antibodies (Fig. 2).

To demonstrate that FIT effectively measures the antibodies directed against epitopes recognized by each FLAG-Fab, the same analysis was performed on mock specimens prepared by spiking negative sera with human monoclonal antibodies of given specificity, obtaining mock samples containing known amounts of IgG directed against the HCV/E2 epitopes defined by two of our Fabs. Results (Fig. 3) showed a linear correlation between the binding of labeled Fabs and the amount of corresponding IgG antibody present in the mock specimen, indicating that FIT can provide reliable information on the amount of epitope-specific antibodies in a patient's serum.

On the assumption that FIT measures the amount of antibodies recognizing the same epitopes recognized by our anti-HCV/E2 human monoclonal recombinant Fabs, we used this method to demonstrate whether such antibodies are elicited in patients infected with HCV of genotypes other than 1a and 1b. For all the epitopes studied in our work, FIT was consistently positive in most HCV-infected sera with a wide range of dilutions. FIT was very diverse for the different epitopes in the same serum sample, with considerable heterogeneity between patients. A summary of FIT values against different Fabs and information on patients and viral genotypes is reported in Table 1, showing no predictable correlation between the total anti-E2 titer and the amount of antibodies directed against single epitopes as measured by FIT. With a view to

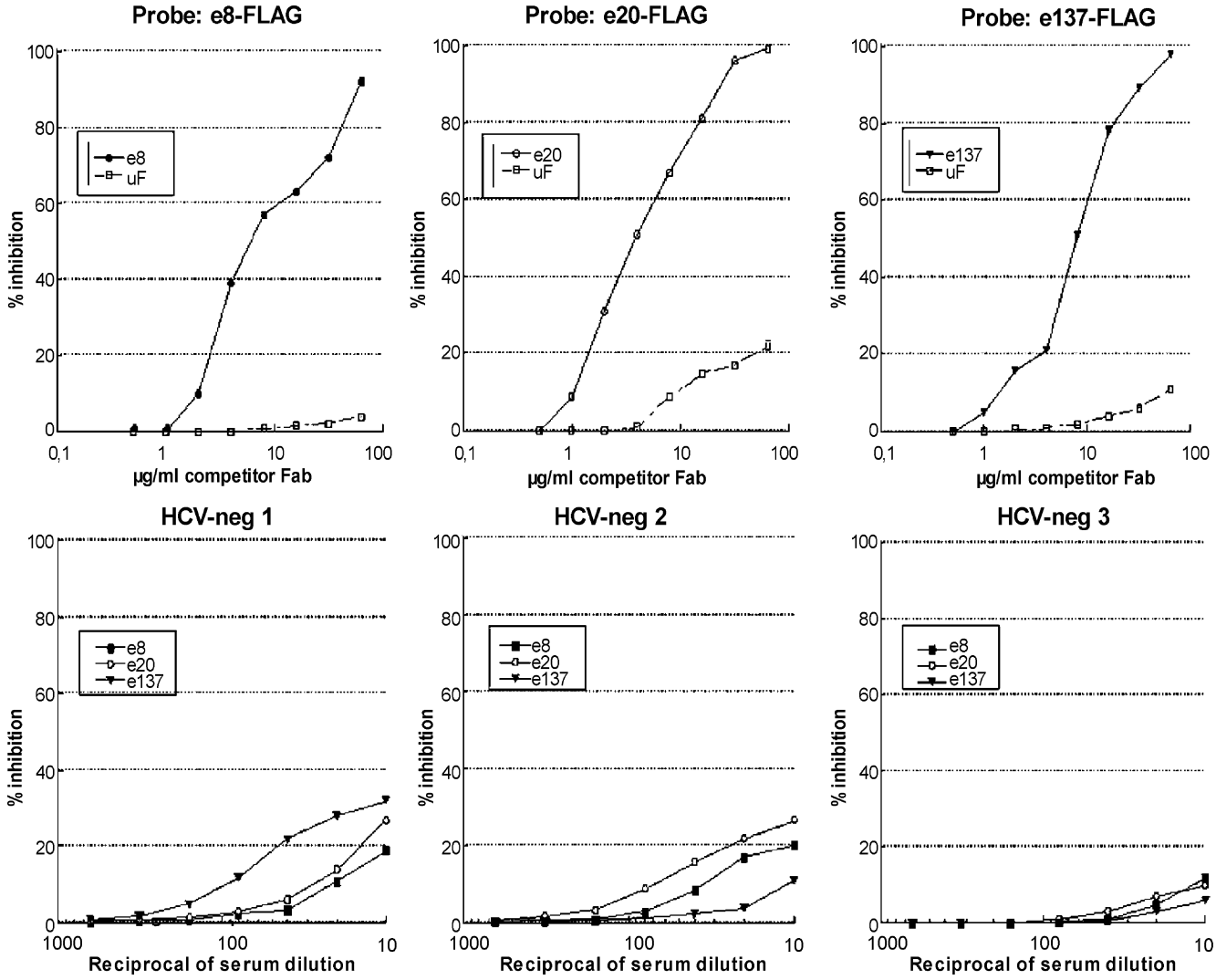

Fig. 2. Competition with unlabeled Fabs and HCV-negative sera. In the upper row, the FLAG-Fabs (e8; e20; e137) used as probes in this study are competed with unlabeled Fabs directed against the same epitopes and with uninfluential Fabs (μF). In the lower row, FIT determination on three different HCV-negative sera is represented.

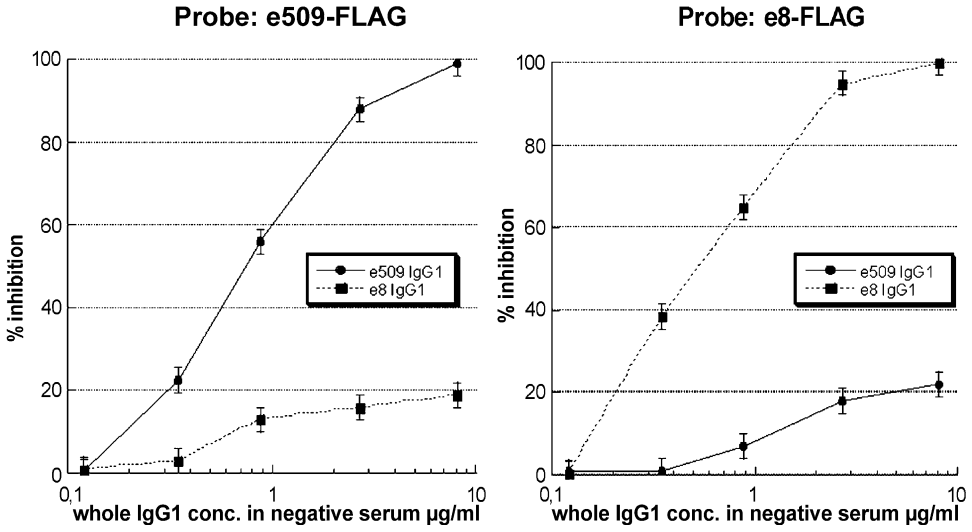

Fig. 3. Competition with mock HCV-positive sera. The validation of FIT was performed on artificial HCV-positive sera with a given amount of characterized anti-HCV/E2 antibodies. In A, the specific probe (e509-FLAG) is inhibited only by the serum containing an increasing amount of the same antibody as whole IgG1 (e509 IgG1). No significant inhibition is evidenced, even at the highest concentration, with the serum containing an antibody (e8 IgG1) directed against a different epitope. In B, data obtained with e8-FLAG as probe are reported.

ruling out the possibility of a negative FIT due to antigen conformational changes induced by other antibodies, we spiked FIT-negative sera with known amounts of recombinant Ig; these mock specimens exhibited restored FIT activity corresponding with the Ig concentration used (data not shown).

In this paper, we describe a new method for assessing the presence in patients' sera of antibodies able to inhibit human recombinant Fab binding to the E2/HCV glycoprotein and its use in demonstrating that all HCVs are able to elicit cross-reactive and HCV/vesicular stomatitis virus (VSV) pseudotype-neutralizing antibodies. The test is specific, as

Table 1
Characteristics of the HCV-positive sera used in this study

Serum	Genotype	Viral load (IU/ml)	Titer on HCV/E2-1a	FIT		
				e8	e20	e137
HCV genotype 1						
1	1a	5,724,000	1:5120	160	20	<10
2	1b	1,197,000	1:10,240	40	80	80
3	1a	57,000	1:2560	20	80	320
4	1b	49,000	1:5120	<10	80	40
5	1b	1,343,000	1:5120	<10	640	160
6	1b	389,000	1:5120	80	80	20
7	1b	606,000	1:2560	40	160	20
8	1a	2,495,000	1:10,240	320	160	<10
9	1b	1,038,000	1:5120	160	640	160
10	1a	20,000	1:20,480	640	640	640
11	1b	405,000	1:1280	160	80	40
12	1a	4,162,000	1:20,480	640	640	80
HCV genotypes other than 1						
13	3a	1,000,000	1:20,480	2560	2560	640
14	3a	751,000	1:640	<10	20	<10
15	2a/2c	605,000	1:20,480	<10	320	640
16	3a	966,000	1:2560	20	80	40
17	3a	1,050,000	1:10,240	80	320	320
18	3a	276,000	1:5120	40	160	80
19	4c/4d	397,000	1:10,240	320	1280	320
20	3a	1,060,000	1:5120	40	640	640
21	2a/2c	680,000	1:2560	<10	80	20
22	2a/2c	ND	1:5120	40	160	20
23	3a	1,026,530	1:10,240	640	640	640
24	2a/2c	882,219	1:2560	80	640	80

demonstrated by the absence of inhibition by sera of HCV-negative subjects. In addition, mock specimens were used to demonstrate that FIT is related to the amount of serum antibodies competing with our Fabs. The possibility that viral glycoproteins bound to an ELISA well (or presented on the surface of a pseudotype virion) could not have the same conformation as the genuine HCV particle does remain; however, all the anti-E2 human monoclonal antibodies used in this study have been elicited by a natural infection, so—at least from a theoretical standpoint—they should recognize viral proteins in their native conformation. However, because large amounts of purified clonal virus are not available, this cannot be proved materially. This assay provides an indirect measure of the amount of antibodies directed against the different human B epitopes present in the E2 molecule and recognized by our Fabs. As these epitopes are not reproducible by synthesizing synthetic peptides (Burioni et al., 1998c), this is at present the only way to determine the amount of antibodies against conserved conformational epitopes of E2. Analysis of several sera from patients persistently infected with HCV indicated how antibodies corresponding with the clones previously studied and shown to have a neutralizing effect on an HCV/VSV pseudovirus model are elicited by interaction of the human immune system with HCVs of different genotypes. Though little is still known about the role that antibodies play during HCV infection, anti-E2 antibodies per se are known to afford no protection and to have no association with a better prognosis. In effect, there was no predictable correlation between FIT against a given epitope and overall anti-E2 serum titer; this may explain the lack of protection even in the presence of a strong anti-E2 antibody response. With the method described above, the different populations of the antibodies that recognize different parts of the E2 molecule can now be measured and their level correlated with clinical and epidemiological data.

The present study does not address the question whether a particular FIT in a given serum correlates with a better prognosis or with given clinical features, nor whether a higher FIT corresponds with a higher pseudovirus-neutralizing titer of the serum, which could be the sum of the activities of all the antibody clones elicited by the virus. The evaluation of the biological activity of our monoclonal antibodies, and as a consequence also of the serum antibodies we measure, depends strongly on the availability of novel and more efficient HCV neutralization models. However, our method in conjunction with more accurate neutralization assays and with the determination of viral sequences in selected patients, for example, patients with spontaneously resolving infection or newborns—where the immune pressure seems to be crucial in driving viral escape mutants emergence (Manzin et al., 2000)—can be very useful in elucidating the virus–host interplay and in shedding light on the emergence of viral variants escaping the immune system.

In conclusion, the FIT test described in this paper is a fast and high-throughput assay able to measure the amount of antibodies directed against discrete HCV/E2 epitopes. Using this novel assay, we were able to demonstrate that cross-reactive antibodies binding to the same epitopes recognized by HCV/VSV pseudovirus-neutralizing human monoclonal antibodies are present after infection due to a broad variety of virus variants. The antibody response against the major HCV glycoprotein, considered the most important viral structure interacting with cellular targets in the initiation of HCV infection, can now be dissected and studied to a deeper level, obtaining new information on the complex interplay between antibodies and virus.

Materials and methods

Anti HCV/E2 human Fabs, FLAG-Fabs, and monoclonal antibodies

Generation, purification, and characterization of the anti-HCV/E2 Fabs used in this study have been described (Burioni et al., 1998c). FLAG-Fabs (Fabs labeled with a FLAG epitope fused at the carboxyterminal of the heavy chain fragment with a pentapeptide bridge) were constructed and purified as described (Burioni et al., 2001a, 2001b). Briefly, FLAG-Fabs were produced using the pComb3/FLAG vector, derived from pComb3/CAF, an expression vector retaining the *Spe*I restriction site 3' of the heavy chain fragment (Burioni et al., 1998a). A synthetic DNA fragment coding for a gly-gln-ala-gly-pro-(FLAG) peptide (Hopp et al., 1988) was used to replace the *Spe*I–*Nhe*I fragment of pComb/CAF, thus generating—with the correct orientation—a new vector (pComb3/FLAG) expressing the recombinant Fab fused to the FLAG epitope, which is recognized by a mouse monoclonal antibody (anti-FLAG M2, Sigma, Saint Louis, MO). Fabs and FLAG-Fabs used in this study were produced by cloning the genes coding for the desired Fab heavy and light chain fragments into pComb3/CAF and pComb3/FLAG, respectively. The Fab was transferred from the pComb3/CAF vector to the pComb3/FLAG vector by cutting pComb3/CAF with *Spe*I–*Sac*I or *Spe*I–*Eco*RI (depending on the presence of the *Sac*I or the *Eco*RI site in the heavy chain fragment sequence) and ligating the corresponding fragment in the identically cut pComb3/CAF containing the genes coding for the desired Fab. This procedure transformed the pComb3/CAF vector in a pComb3/FLAG vector, producing FLAG-Fabs. Phagemid-transformed *E. coli* XL-1 Blue was used as a source for Fab production in bacterial cultures. For this purpose, a single colony obtained from transformation was inoculated into 10 ml SB (Barbas et al., 1991) containing ampicillin (50 μg/ml); after 8 h of incubation at 37 °C, isopropyl-beta-D-thio-galactopyranoside was added to a final concentration of 1 mM and cells were incubated for an additional 14 h at 30 °C in a rotatory shaker. Bacteria

were harvested by centrifugation at 5000 × *g* for 20 min, resuspended in 1 ml of phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA, Sigma), and lysed by a freeze–thawing procedure (Williamson et al., 1993). Cell debris was removed by centrifugation in a microfuge at 15,000 × *g* for 5 min at room temperature and supernatants were used in ELISA experiments. When necessary, Fab and FLAG-Fab were purified by immunoaffinity and quantified as described (Barbas et al., 1992).

Standardization of the assay was performed using Fab-coding genes to construct full-size human monoclonal antibodies (HuMabs), which were inserted in an appropriate eukaryotic vector for subsequent production in transfected CHO-K1 cells as described (Burton et al., 1994). The HuMabs present in the culture supernatant were purified by immunoaffinity (Barbas et al., 1992) and purity-checked by PAGE. The amount of human antibody was assayed by a sandwich immunoassay (Burton et al., 1994). All antibodies and Fabs were stored at −70 °C until use. Sera obtained from healthy donors and HCV-positive patients were tested using commercial kits for anti-HCV antibody (Vitros Anti-HCV assay; Ortho, Raritan, NJ, USA), viral load (Cobas Amplicor HCV monitor assay 2.0; Roche Molecular Diagnostics, Tokyo, Japan), and viral genotype (INNO-LiPA HCV II test; Innogenetics, Ghent, Belgium). For the preparation of mock specimens with known amounts of antibodies directed against a given epitope, HCV-negative sera were spiked with concentrated purified human monoclonal antibodies in PBS. From this point on, mock specimens were treated exactly like the positive and negative sera.

Fab inhibition titer (FIT) assay

The purpose of the FIT assay is to assess the ability of sera to inhibit the binding of a labeled Fab to its epitope, thus obtaining an indirect measure of the amount of epitope-binding serum antibodies. From the practical point of view, as a first step FLAG-Fabs were purified and assayed in a FLAG-Fab-specific ELISA (Burioni et al., 2001a) to determine the correct concentration to be used in inhibition experiments. Briefly, FLAG-Fab preparations of known concentration were titered by ELISA (Williamson et al., 1993) using plates coated overnight at 4 °C with recombinant HCV/E2 (genotype 1a, 100 ng/well in 25 μl of coating buffer) produced in CHO cells (Lesniewski et al., 1995). Plates were blocked for 1 h at 37 °C with PBS/1%BSA. After removing the blocking solution, 50 μl of progressive dilutions of FLAG-Fab made in PBS/BSA 1% was added to the wells and incubated for 2 h at 37 °C. Plates were washed 10 times with PBS/0.05% Tween 20 (Sigma) in an automated plate washer (Sorin, Saluggia, Italy) before adding 50 μl of a 10 μg/ml solution of anti-FLAG mouse monoclonal antibody M2 (Sigma) in PBS/BSA 1%. After 1-h incubation at 37 °C, wells were washed 10 times with PBS/Tween 20 as above and mouse monoclonal binding was revealed with horseradish peroxidase-conjugated goat anti-mouse IgG (1:10,000 in PBS/BSA 1%; Pierce, Rockford, IL). Substrate was added and plates were read for OD_{450} in an automated plate reader after 30-min incubation at room temperature in the dark. All assays were performed at least in double. A negative control antigen (BSA) was always included and the OD reading was subtracted as background.

Determination of FIT in human sera

For the determination of the FIT of sera, a concentration of purified FLAG-Fabs yielding in standard conditions an OD_{450} reading equal to 50% of maximum reading was used for further experiments of Fab inhibition ELISA. For these experiments, plates were coated and blocked as described above. Progressive 1:2 serum dilutions starting at 1:10 in PBS/BSA 1% were added in the amount of 50 μl per ELISA well. After 2 h of incubation at 37 °C, purified FLAG-Fab was added directly to serum dilutions to reach the desired final concentration. Plates were incubated for an additional 30 min and then processed as described above for FLAG-Fab ELISA. A positive control sample, containing a 20:1 excess of purified unlabeled Fab, corresponding with 100% inhibition, was included. A negative control sample, containing an excess of a control uninfluential Fab (Burioni et al., 1998b) and corresponding with 0% inhibition, was also included. The final results were determined as percent of inhibition with the formula: percent inhibition = 100 × (OD_{450} of probe FLAG-Fab alone − OD_{450} of probe FLAG-Fab with competing serum)/OD_{450} of probe FLAG-Fab alone. The highest serum dilution giving more than 50% inhibition of FLAG-Fab binding was considered as the FIT for that epitope and serum.

Moreover, anti-HCV/E2 titer was determined for each serum. ELISA plates were coated and blocked as above and 50 μl of 1:2 progressive serum dilutions in PBS/BSA 1% was added to the wells and incubated for 2 h at 37 °C. Bound serum antibodies were revealed by adding 50 μl of horseradish peroxidase-conjugated goat antihuman IgG Fc fragment (1:6000 in PBS/BSA1%; Sigma). A negative control antigen (BSA) was also added. As titering dilution, the highest giving a significantly higher (differential OD_{450} > 0.3) signal on HCVE2 than on control antigen was chosen.

Acknowledgments

This work was financed by grants from Istituto Superiore di Sanità-AIDS to R.B and M.C.

The pIgG1 vector was kindly provided by Dennis R. Burton, The Scripps Institute, La Jolla, CA. The authors thank Silvio Bighi, Eleonora Berardinelli, Giorgia Serafini, and Giuseppe Malcangi for their assistance.

References

Alter, H.J., Purcell, R.H., Shih, J.W., Melpolder, J.C., Houghton, M., Choo, Q.L., Kuo, G., 1989. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N. Engl. J. Med. 321 (22), 1494–1500.

Alter, M.J., Margolis, H.S., Krawczynski, K., Judson, F.N., Mares, A., Alexander, W.J., Hu, P.Y., Miller, J.K., Gerber, M.A., Sampliner, R.E., et al., 1992. The natural history of community-acquired hepatitis C in the United States. The sentinel counties chronic non-A, non-B hepatitis study team. N. Engl. J. Med. 327 (27), 1899–1905.

Barbas, C.F.d., Kang, A.S., Lerner, R.A., Benkovic, S.J., 1991. Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. Proc. Natl. Acad. Sci. U.S.A. 88 (18), 7978–7982.

Barbas, C.F.d., Bjorling, E., Chiodi, F., Dunlop, N., Cababa, D., Jones, T.M., Zebedee, S.L., Persson, M.A., Nara, P.L., Norrby, E., et al., 1992. Recombinant human Fab fragments neutralize human type 1 immunodeficiency virus in vitro. Proc. Natl. Acad. Sci. U.S.A. 89 (19), 9339–9343.

Bjoro, K., Froland, S.S., Yun, Z., Samdal, H.H., Haaland, T., 1994. Hepatitis C infection in patients with primary hypogammaglobulinemia after treatment with contaminated immune globulin. N. Engl. J. Med. 331 (24), 1607–1611.

Bugli, F., Mancini, N., Kang, C.Y., DiCampli, C., Grieco, A., Manzin, A., Gabrielli, A., Gasbarrini, A., Fadda, G., Varaldo, P.E., Clementi, M., Burioni, R., 2001. Mapping B cell epitopes of hepatitis C virus E2 glycoprotein using human monoclonal antibodies from phage display libraries. J. Virol. 75 (20), 9986–9990.

Burioni, R., Plaisant, P., Bugli, F., Delli Carri, V., Clementi, M., Fadda, G., 1998a. A vector for the expression of recombinant monoclonal Fab fragments in bacteria. J. Immunol. Methods 217 (1–2), 195–199.

Burioni, R., Plaisant, P., Bugli, F., Solforosi, L., Carri, V.D., Varaldo, P.E., Fadda, G., 1998b. A new subtraction technique for molecular cloning of rare antiviral antibody specificities from phage display libraries. Res. Virol. 149 (5), 327–330.

Burioni, R., Plaisant, P., Manzin, A., Rosa, D., Delli Carri, V., Bugli, F., Solforosi, L., Abrignani, S., Varaldo, P.E., Fadda, G., Clementi, M., 1998c. Dissection of human humoral immune response against hepatitis C virus E2 glycoprotein by repertoire cloning and generation of recombinant Fab fragments. Hepatology 28 (3), 810–814.

Burioni, R., Bugli, F., Mancini, N., Fadda, G., 2001a. A novel expression vector for production of epitope-tagged recombinant Fab fragments in bacteria. Hum. Antibodies 10 (3,4), 149–154.

Burioni, R., Bugli, F., Mancini, N., Rosa, D., DiCampli, C., Moroncini, G., Manzin, A., Abrignani, S., Varaldo, P.E., Clementi, M., Fadda, G., 2001b. Non-neutralizing human antibody fragments against hepatitis C virus E2 glycoprotein modulate neutralization of binding activity of human recombinant Fabs. Virology 288, 29–35.

Burioni, R., Matsuura, Y., Mancini, N., Tani, H., Miyamura, T., Varaldo, P.E., Clementi, M., 2002. Diverging effects of human recombinant anti-hepatitis C virus (HCV) antibody fragments derived from a single patient on the infectivity of a vesicular stomatitis virus/HCV pseudotype. J. Virol. 76 (22), 11775–11779.

Burton, D.R., Pyati, J., Koduri, R., Sharp, S.J., Thornton, G.B., Parren, P.W., Sawyer, L.S., Hendry, R.M., Dunlop, N., Nara, P.L., Lamacchia, M., Garratty, E., Stiehm, E.R., Bryson, J.Y., Cao, Y., Moore, J.P., Ho, D.D., Barbas, C.F., 1994. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science 266 (5187), 1024–1027.

Cerny, A., Chisari, F.V., 1999. Pathogenesis of chronic hepatitis C: immunological features of hepatic injury and viral persistence. Hepatology 30 (3), 595–601.

Farci, P., Alter, H.J., Govindarajan, S., Wong, D.C., Engle, R., Lesniewski, R.R., Mushahwar, I.K., Desai, S.M., Miller, R.H., Ogata, N., et al., 1992. Lack of protective immunity against reinfection with hepatitis C virus. Science 258 (5079), 135–140.

Farci, P., Alter, H.J., Wong, D.C., Miller, R.H., Govindarajan, S., Engle, R., Shapiro, M., Purcell, R.H., 1994. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. Proc. Natl. Acad. Sci. U.S.A. 91 (16), 7792–7796.

Hopp, T.P., Prickett, K.S., Price, V.L., Libby, R.T., March, C.J., Cervetti, D.P., Urdal, D.L., Corbon, P.J., 1988. A short polypeptide marker sequence useful for recombinant protein identification and purification. Bio/Technology 6, 1204–1210.

Lai, M.E., Mazzoleni, A.P., Argiolu, F., De Virgilis, S., Balestrieri, A., Purcell, R.H., Cao, A., Farci, P., 1994. Hepatitis C virus in multiple episodes of acute hepatitis in polytransfused thalassaemic children. Lancet 343 (8894), 388–390.

Lauer, G.M., Walker, B.D., 2001. Hepatitis C virus infection. N. Engl. J. Med. 345 (1), 41–52.

Lesniewski, R., Okasinski, G., Carrick, R., Van Sant, C., Desai, S., Johnson, R., Scheffel, J., Moore, B., Mushahwar, I., 1995. Antibody to hepatitis C virus second envelope (HCV-E2) glycoprotein: a new marker of HCV infection closely associated with viremia. J. Med. Virol. 45 (4), 415–422.

Manzin, A., Solforosi, L., Debiaggi, M., Zara, F., Tanzi, E., Romano, L., Zanetti, A.R., Clementi, M., 2000. Dominant role of host selective pressure in driving hepatitis C virus evolution in perinatal infection. J. Virol. 74 (9), 4327–4334.

Ogata, N., Alter, H.J., Miller, R.H., Purcell, R.H., 1991. Nucleotide sequence and mutation rate of the H strain of hepatitis C virus. Proc. Natl. Acad. Sci. U.S.A. 88 (8), 3392–3396.

Piazza, M., Sagliocca, L., Tosone, G., Guadagnino, V., Stazi, M.A., Orlando, R., Borgia, G., Rosa, D., Abrignani, S., Palumbo, F., Manzin, A., Clementi, M., 1997. Sexual transmission of the hepatitis C virus and efficacy of prophylaxis with intramuscular immune serum globulin. A randomized controlled trial. Arch. Intern. Med. 157 (14), 1537–1544.

Plaisant, P., Burioni, R., Manzin, A., Solforosi, L., Candela, M., Gabrielli, A., Fadda, G., Clementi, M., 1997. Human monoclonal recombinant Fabs specific for HCV antigens obtained by repertoire cloning in phage display combinatorial vectors. Res. Virol. 148 (2), 165–169.

Williamson, R.A., Burioni, R., Sanna, P.P., Partridge, L.J., Barbas, C.F.d., Burton, D.R., 1993. Human monoclonal antibodies against a plethora of viral pathogens from single combinatorial libraries. Proc. Natl. Acad. Sci. U.S.A. 90 (9), 4141–4145.

Zibert, A., Schreier, E., Roggendorf, M., 1995. Antibodies in human sera specific to hypervariable region 1 of hepatitis C virus can block viral attachment. Virology 208 (2), 653–661.